

**LISTING OF CLAIMS**

This listing of claims will replace all prior versions, and listings, of claims in the application.

**Listing of Claims**

1-28. (CANCELED)

29. (CURRENTLY AMENDED) A method for isolating purified RNA from a biological sample comprising

a) treating the biological sample with a reagent comprising phenol that when added to the biological sample at a final phenol concentration is from about 10%<sup>w/w</sup> to about 60%<sup>w/w</sup> and at least one ribonuclease inhibitor,

b) mixing the mixture containing biological sample from step (a) with at least one hydrophobic solvent and a buffer at a concentration sufficient to maintain a pH in the range from about pH 3.6 to below pH 4.0,

c) separating an aqueous phase[[s]] from the mixture obtained in step (b) by sedimentation and recovering purified RNA from an aqueous phase by precipitation with about an equal volume of a water-soluble organic solvent which is RNA that does not reveal the presence of DNA when assayed by reverse transcription polymerase chain reaction (RT-PCR), and

d) washing and solubilizing the precipitated RNA.

30. (ORIGINAL) The method of claim 29 wherein the reagent in (a) further comprises a buffer selected from at least one of acetate, citrate, phosphate, phthalate, tartrate, lactate, or mixtures thereof.

31. (CANCELED)

32. (CURRENTLY AMENDED) The method of claim ~~34~~ 29 wherein the ribonuclease inhibitor is selected from at least one of proteinase K, ribonuclease inhibitor from human placenta, vanadyl ribonucleoside complex, chaotropic salts, or mixtures thereof.

33. (ORIGINAL) The method of claim 32 wherein the chaotropic salts are selected from at least one of urea salts, guanidine salts, or mixtures thereof.

34. (ORIGINAL) The method of claim 33 wherein the guanidine salts are selected from at least one of guanidine thiocyanate or guanidine hydrochloride at a final concentration in the range of about 0.5 M to about 6 M.

35. (PREVIOUSLY PRESENTED) The method of claim 29 wherein the reagent in (a) further comprises a detergent at a concentration  $< 0.1\%^{w/w}$ .

36. (ORIGINAL) The method of claim 35 wherein the detergent is selected from at least one of sarcosine, polyoxyethylenesorbitan, a dodecylsulfate salt, or mixtures thereof.

37. (ORIGINAL) The method of claim 29 wherein the reagent in (a) further comprises an inorganic or organic salt and a chelating agent.

38. (ORIGINAL) The method of claim 37 wherein the inorganic or organic salt is selected from at least one of chlorides, phosphates, bromates, acetates, citrates, phthalates, tartrates, lactates, or thiocyanates of sodium, potassium, lithium or ammonium.

39. (ORIGINAL) The method of claim 37 wherein the chelating agent is selected from at least one of citrates, ethylenediamine tetraacetic salts, or mixtures thereof.

40. (CANCELED)

41. (ORIGINAL) The method of claim 29 wherein the reagent in (a) further comprises phenol solubilizers selected from at least one of polyalcohols, monoalcohols, and guanidine salts.

42-43. (CANCELED)

44. (PREVIOUSLY PRESENTED) A method for isolating purified RNA from a biological sample comprising

- a) treating the sample with a phenol-free composition comprising
  - at least one hydrophobic organic solvent at a final concentration in the range from about 10%<sup>w/w</sup> to about 40%<sup>w/w</sup>, and at least one acid sufficient to maintain a pH in the range of about pH 3.6 to below pH 4.0 during phase separation, and an optional acid solubilizer, or
  - at least one ribonuclease inhibitor and a buffer selected from at least one of acetate, citrate, phosphate, phthalate, tartrate, lactate, or mixtures thereof, sufficient to maintain a pH of the composition in the range from about pH 3.6 to below pH 4.0;

b) then treating the sample with a reagent comprising phenol at a final concentration ranging from about 10%<sup>w/w</sup> to about 60%<sup>w/w</sup> and at least one ribonuclease inhibitor;

c) mixing the sample with at least one hydrophobic solvent while maintaining a pH in the range from about pH 3.6 to below pH 4.0;

d) recovering purified RNA from an aqueous phase to which about an equal volume of a water-soluble organic solvent is added to precipitate the purified RNA which is RNA that does not reveal the presence of DNA when assayed by reverse transcription polymerase chain reaction (RT-PCR); and

e) washing and solubilizing the precipitated RNA.

45. (CANCELED)

46. (ORIGINAL) The method according to claims 29 or 44 wherein step (a) is performed at a pH ranging from about pH 3.9 to about pH 9.0, and the sample is then adjusted to a pH ranging from about pH 3.6 to below pH 4.0.

47. (Currently Amended) An acidic phenol precipitation method for isolating purified RNA from a biological sample comprising the steps of

a) treating the biological sample with a mono-phase reagent comprising phenol at a final concentration ranging from about 3%<sup>w/w</sup> to less than 30%<sup>w/w</sup> and a buffer sufficient to maintain a pH of the resulting mixture containing biological sample in the range from about pH 3.6 to about pH 5.5,

b) sedimenting or filtering the mixture containing biological sample to obtain a purified biological sample substantially free of DNA, proteins, and cellular components without the use of a

hydrophobic solvent and performing phase separation and wherein the pH of the mixture containing biological sample remains in the range from about pH 3.6 to about pH 5.5,

- c) adding to the purified biological sample about an equal volume of a water-soluble organic solvent to precipitate purified RNA which is RNA that does not reveal the presence of DNA when assayed by reverse transcription polymerase chain reaction (RT-PCR),
- d) sedimenting or filtering the precipitated RNA, and
- e) washing and solubilizing the precipitated RNA.

48. (PREVIOUSLY PRESENTED) A two-step method for isolating purified RNA from a biological sample comprising

a) treating the biological sample with a mono-phase reagent comprising phenol at a final concentration ranging from about 3%<sup>w/w</sup> to less than 30%<sup>w/w</sup>, at least one chaotrope, and a buffer sufficient to maintain a pH of the resulting mixture containing biological sample in the range from about pH 3.6 to about pH 5.5,

b) sedimenting or filtering the mixture containing biological sample to obtain a purified biological sample substantially free of DNA, proteins, and cellular components,

c) adding to the purified biological sample at least one hydrophobic organic solvent and a buffer in a concentration sufficient to maintain a pH of the purified biological sample in the range from about pH 3.6 to below pH 4.0,

d) recovering purified RNA from an aqueous phase to which about an equal volume of a water soluble organic solvent is added to precipitate purified RNA which is RNA that does not reveal the presence of DNA when assayed by reverse transcription polymerase chain reaction (RT-PCR),

e) sedimenting or filtrating the precipitated RNA, and

f) washing and solubilizing the precipitated RNA.

49. (ORIGINAL) The method of claim 48 where the hydrophobic organic solvent is sufficiently dense to separate the organic phase during phase separation.

50. (PREVIOUSLY PRESENTED) The method according to claim 48 wherein the hydrophobic organic solvent is selected from at least one of caprolactone, ethylene glycol diacetate, polyethylene glycol dibenzoate, chloroform, carbon tetrachloride, bromochloropropane, bromonaphthalene, bromoanisole, or mixtures thereof.

51. (ORIGINAL) The method according to claims 47 or 48 wherein the sample is treated with the composition of (a) at about 1.5X to about 2.5X concentration, and the resulting sample is diluted to approach the non-concentrated solution.

52. (ORIGINAL) The method according to any of claims 29, 44, 47, or 48 wherein the solvent added to precipitate RNA is at least one of lower alcohols, polyalcohols, acetone, ethyleneglycol diacetate, methyl sulfoxide, or mixtures thereof.

53-58. (CANCELED)

59. (CURRENTLY AMENDED) A method for selectively precipitating higher molecular weight RNA from a biological sample comprising

treating the biological sample with an aqueous composition comprising phenol at a final concentration ranging from about 1%<sup>w/w</sup> to about 60%<sup>w/w</sup>, at least one chaotrope, a buffer in a concentration sufficient to maintain a pH of the composition in the range from about pH 2.0 to about pH 9.0, at least one water-soluble organic solvent at a concentration from about 10%<sup>w/w</sup> to about 40%<sup>w/w</sup> to selectively precipitate ~~higher molecular weight RNA~~ molecules greater than 200 based from the biological sample, and

precipitating purified higher molecular weight RNA from the biological sample.

60. (ORIGINAL) The method of claim 59 further comprising the step of thereafter adding additional organic solvent sufficient to increase the concentration of organic solvent to at least 50%<sup>w/w</sup> to precipitate lower molecular weight RNA, and precipitating purified lower molecular weight RNA from the sample.

61. (ORIGINAL) The method of claim 59 comprising preparing the biological sample according to any of claims 29, 44, 47, or 48 to obtain an aqueous solution of RNA, and precipitating RNA from the aqueous solution.

62. (PREVIOUSLY PRESENTED) A method for isolating purified RNA from a biological sample comprising

a) treating the sample with a reagent comprising phenol at a final concentration ranging from about 10%<sup>w/w</sup> to about 60%<sup>w/w</sup> and at least one ribonuclease inhibitor, the phenol comprising derivative selected from at least one of phenylethanol, propylene phenoxylol, thymol, butylphenol, or mixtures thereof at a final concentration up to about 5%<sup>w/w</sup>,

b) mixing the sample with at least one hydrophobic solvent while maintaining a pH in the range from about pH 3.6 to below pH 4.0,

c) recovering purified RNA from an aqueous phase to which about an equal volume of a water-soluble organic solvent is added to precipitate the purified RNA, and

d) washing and solubilizing the precipitated RNA.

63. (PREVIOUSLY PRESENTED) A method for isolating purified RNA from a biological sample comprising

a) treating the sample with a reagent comprising phenol at a final concentration ranging from about 10%<sup>w/w</sup> to about 60%<sup>w/w</sup>, at least one ribonuclease inhibitor, and an organic compound selected from at least one of cyclohexyl bromide, dibromopropane, dichlorobenzoic acid, and mixtures thereof in a concentration ranging from about 1%<sup>w/w</sup> to about 5%<sup>w/w</sup> sufficient to increase the density of the composition,

b) mixing the sample with at least one hydrophobic solvent while maintaining a pH in the range from about pH 3.6 to below pH 4.0,

c) recovering purified RNA from an aqueous phase to which about an equal volume of a water-soluble organic solvent is added to precipitate the purified RNA, and

d) washing and solubilizing the precipitated RNA.